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Genetic recombination in bacteria: horizon of the beginnings of sexuality in living organisms

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Abstract. The current paper reviews the bacterian genetic recombination. Bacteria can transfer genes from one strain to another by three different mechanisms: transformation, conjugation and transduction, these events showing the universality of sexuality in the living world. Besides genetic recombination in bacteria, recent evidences of genetic recombination in some superior animals (such as: fish, birds, mammals and humans) at the sex-chromosomes level support the 'gene concept of sexuality' as a general view of sexuality.
Key words: transformation, conjugation, transduction, bacteria, genetic recombination, sexuality.

Introduction. Bacteria is the most extremophilic and diversified group of organisms on Earth (Gagyi-Palffy & Stoian 2008), and they are crucial to the maintenance of Earth's environment (Coșier & Petrescu-Mag 2008). Various species release oxygen into the atmosphere; recycle carbon, nitrogen (Carpa & Butiuc-Keul 2009) and other elements and digest human and other animal wastes (Bodoczi 2009) as well as pesticides and other pollutants, which would otherwise eventually poison the air, soil and water. Bacteria also cause hundreds of animal and plant diseases but even so, harmful species are a small fraction of all bacteria. Many species, in fact, produce vitamins and other materials essential to the health and survival of humans and other organisms. It is important to mention here that bacteria are the most studied models of extraterrestrial life (see Movile Cave from Romania, Pricop & Negrea 2009; or Dobrotă & Carpa 2009).

Bacteria have genes composed of DNA arranged in a long series on a "chromosome". Their genetic material is not organized in the same way as that of eukariotes. They belong to a class of organisms known as prokariotes. Two key features of bacteria are their astounding ability to proliferate and their enormous diversity. Hence, even though bacteria and phages do not undergo meiosis, the approach to the genetic analysis of these forms is surprisingly similar to that for eukaryotes. Bacteria have no sexual reproduction in the sense that eukaryotes do. The have no alternation of diploid and haploid generations, no gametes and no meiosis. But the essence of sex is genetic recombination, and bacteria do have three mechanisms to accomplish that: transformation, conjugation and transduction.

The opportunity for genetic recombination in bacteria can arise in several different ways, but in all cases two DNA molecules are brought together, and then there must have been some type of "sexual" union. The possibilities are due to gene transfer from one individual to another and it plays an important role in the evolution of new variants in nature. Many cases of such horizontal or lateral gene transfer have come to light through recent molecular and DNA sequencing analyses. Comparative genomic analysis of many different genes in various bacterial species has shown similarities of genes in species thought to be only distantly related. The simplest explanation is that significant transfer of DNA between bacteria occurred throughout evolution. Many researchers can use the various methods of gene transfer to map genes and construct bacterial strains with which to test the function and regulation of specific genes.

Bacteria can transfer genes from one strain to another by three different mechanisms: transformation, conjugation and transduction. In all three mechanisms, one

cell – the donor – provides the genetic material for transfer, while a second cell – the recipient – receives the material. In transformation, DNA from a donor is added to the bacterial growth medium and is then taken up from that medium by the recipient. In conjugation, the donor carries a special type of plasmid that allows it to come in contact with the recipient and transfer DNA directly. In transduction, the donor DNA is packaged within the protein coat of a bacteriophage and trasferred to the recipient when the phage particle infects it. The recipients of a gene transfer are known as transformants, exconjugants or transductans, depending on the mechanism of DNA transfer that created them.

All bacterial gene transfer is asymetrical in two ways. First, transfer goes in only one direction, from donor to recipient. Second, most recipients receive 3% or less of a donor's DNA; only some exconjugants contain a greater percentage of donor material. Thus, the amount of donor DNA entering the recipient is small relative to the size of the recipient's chromosome, and the recipient retains most of its own DNA.

1. Bacterial Transformation. A few species of bacteria spontaneously take up DNA fragments from their surroundings in a process known as natural transformation. The large majority of bacterial species can take up DNA from the surrounding medium only after laboratory procedures make their cell walls and membranes permeable to DNA in a process known as artificial transformation. The DNA taken up integrates into recipient's chromosome.

Researchers have studied several species of bacteria that undergo natural transformation, including *Streptococcus pneumoniae*, the pathogen that caused pneumonia in humans, *B. subtilis*, a harmless soil bacterium, *H. influenzae*, a pathogen causing various diseases in humans, *N. gonorrhoeae* the microbial agent of gonorrhea. Transformation was discovered in the bacterium *Streptococcus pneumoniae* in 1928 by F. Griffith. In 1944, O.T. Avery, C.M MacLeod and M. McCarty demonstrated that the transforming principle was DNA. Both results are milestones in the elucidation of the molecular nature of genes (Lederberg 1994).

Bacteria transformation may be referred to as a stable genetic change brought about by taking up naked DNA (DNA without associated cells or proteins), and competence refers to the state of being able to take up exogenous DNA from the environment. Two different forms of competence should be distinguished: natural and artificial.

Some bacteria (around 1% of all species) are naturally capable of taking up DNA under laboratory conditions; many more may be able to take it up in their natural environments. Such species carry sets of genes specifying the cause of the machinery for bringing DNA across the cell's membrane or membranes (Russi et al 2008).

Artificial competence is not encoded in the cell's genes. Instead it is induced by laboratory procedures in which cells are passively made permeable to DNA, using conditions that do not normally occur in nature (Kunik et al 2001).

Calcium chloride transformation is a method of promoting competence. Chilling cells in the presence of divalent cations such as Ca^{2+} (in $CaCl_2$) prepares the cell membrane to become permeable to plasmid DNA. Cells are incubated on ice with the DNA and then briefly heat shocked (e.g. 42 °C for 30–120 seconds), which causes the DNA to enter the cell. This method works very well for circular plasmid DNAs. An excellent preparation of competent cells will give $\sim 10^8$ colonies per microgram of plasmid. A poor preparation will be about 10^4/μg or less. Good non-commercial preps should give 10^5 to 10^6 transformants per microgram of plasmid (Chen & Dubnau 2004).

The method usually does not work well for linear molecules such as fragments of chromosomal DNA, probably because exonuclease enzymes in the cell rapidly degrade linear DNA. However, cells that are naturally competent are usually transformed more efficiently with linear DNA than with plasmids (Sharma et al 2007).

Electroporation is another way to make holes in bacterial (and other) cells, by briefly shocking them with an electric field of 10-20kV/cm. Plasmid DNA can enter the cell through these holes. This method is amenable to use with large plasmid DNA (Waters

2001). Natural membrane-repair mechanisms will rapidly close these holes after the shock.

Transformation has been a handy tool in several areas of bacterial research because the genotype of a strain can be deliberately changed in a very specific way by transforming with an appropriate DNA fragment. For example, transformation is used widely in genetic engineering (Pimda & Bunnag 2010) (see Figure 1). More recently it has been found that even eukaryotic cells can be transformed, using quite similar procederes, and this technique has been invaluable for modifying eukaryotic cells.

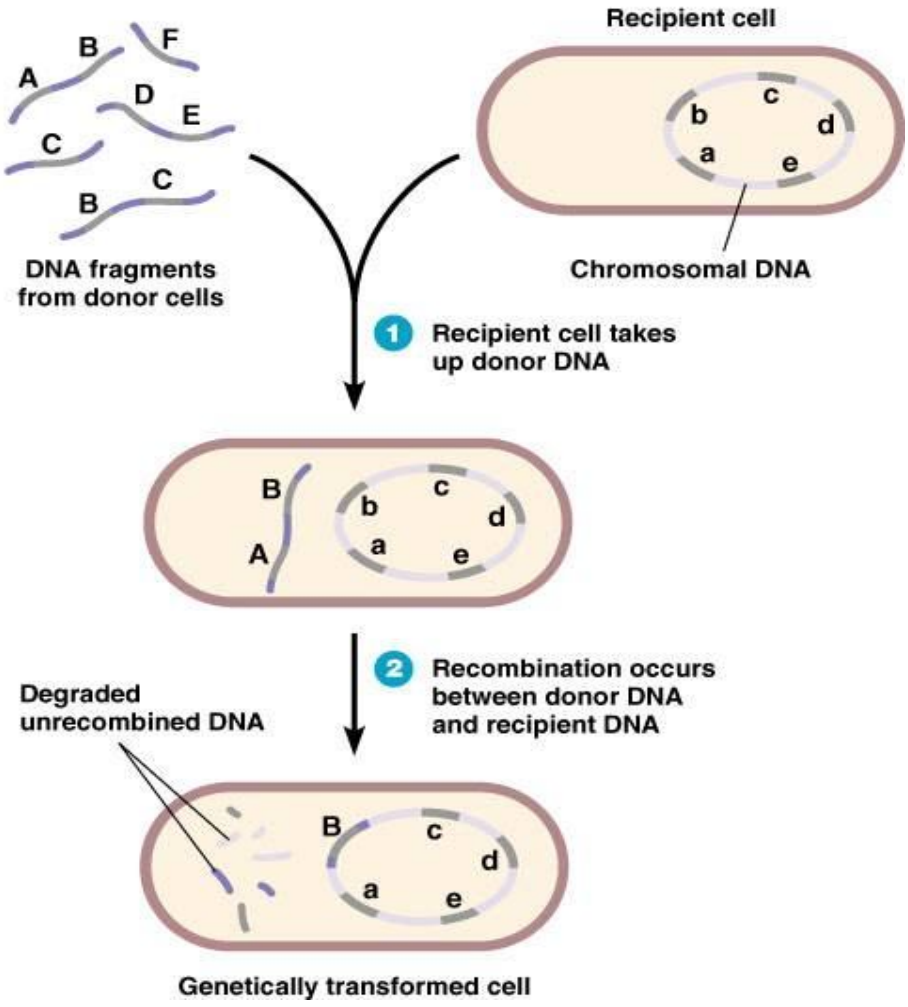

Figure 1. The mechanism of transformation (*)

Transformation can be used to provide information on bacterial gene linkage. When DNA (the bacterial chromosome) is extracted for transformation experiments, some breakage into smaller pieces is inevitable. If two donor genes are located close together on the chromosome, there is a good chance that sometimes they will be carried on the same piece of transforming DNA. Hence, both will be taken up, causing a double transformation. Conversely, if genes are widely separated on the chromosome, they will be carried on separate transforming segments. Any double transformants will most likely arise from separate independent transformations. Hence in the case of widely separated genes, the frequency of double transformants will equal the product of the single-transformation frequencies. Therefore it should be possible to test for close linkage by testing for a departure from the product rule. In other words, if genes are linked, then the proportion of double transformants will be greater then the product of single transformants. Unfortunately, the situation is made more complex by several factors -

the most important is that not all cells in a population of bacteria are competent to be transformed.

2. Bacterial Conjugation. Bacterial conjugation is the transfer of genetic material between bacteria through direct cell to cell contact, or through a bridge-like connection between the two cells (Holmes & Jobling 1996).

Bacterial conjugation is often incorrectly regarded as the bacterial equivalent of sexual reproduction or mating. Loosely, and misleadingly, it can be considered to be a limited bacterial version of sex, since it involves some genetic exchange. In order to perform conjugation, one of the bacteria, the donor, must play host to a conjugative or mobilizable genetic element, most often a conjugative or mobilizable plasmid or transposon (Ryan & Ray 2004). Most conjugative plasmids have systems ensuring that the recipient cell does not already contain a similar element.

The mechanism of conjugation includes: 1- Donor cell produces pilus. 2- Pilus attaches to recipient cell, brings the two cells together. 3- The mobile plasmid is nicked and a single strand of DNA is then transferred to the recipient cell. 4- Both cells recircularize their plasmids, synthesize second strands, and reproduce pili; both cells are now viable donors (see Figure 2).

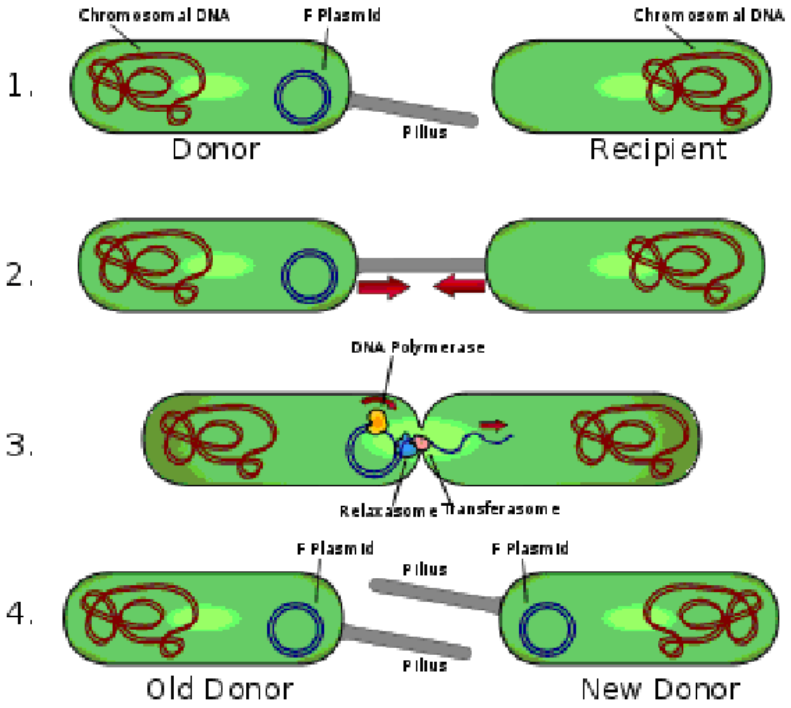

Figure 2. The mechanism of conjugation (**)

The genetic information transferred is often beneficial to the recipient cell. Benefits may include antibiotic resistance, other xenobiotic tolerance, or the ability to utilize a new metabolite. Such beneficial plasmids may be considered bacterial endosymbionts. Some conjugative elements may also be viewed as genetic parasites on the bacterium, and conjugation as a mechanism that was evolved by the mobile element to spread itself into new hosts.

The prototype for conjugative plasmids is the F-plasmid, also called the F-factor. The F-plasmid is an episome (a plasmid that can integrate itself into the bacterial chromosome by homologous recombination) of about 100 kb length. It carries its own origin of replication, the *oriV*, as well as an origin of transfer, or *oriT* (Ryan & Ray 2004). There can only be one copy of the F-plasmid in a given bacterium, either free or integrated (two immediately before cell division). The host bacterium is called F-positive or F-plus (denoted F^+). Strains that lack F plasmids are called F-negative or F-minus (F^-). Among other genetic information, the F-plasmid carries a *tra* and a *trb* locus, which

together are about 33 kb long and consist of about 40 genes. The *tra* locus includes the *pilin* gene and regulatory genes, which together form pili on the cell surface, polymeric proteins that can attach themselves to the surface of F^- bacteria and initiate the conjugation. Several proteins coded for in the *tra* or *trb* loci seem to open a channel between the bacteria. It is thought that the traD enzyme located at the base of the pilus is involved in DNA exchange, by iniating membrane fusion.

When conjugation is initiated, via a mating signal, a relaxase enzyme creates a nick in one plasmid DNA strand at the origin of transfer, or *oriT*. The relaxase may work alone or in a complex of over a dozen proteins, known collectively as a relaxosome. In the F-plasmid system, the relaxase enzyme is called TraI and the relaxosome consists of TraI, TraY, TraM, and the integrated host factor, IHF. The transferred, or *T-strand*, is unwound from the duplex plasmid and transferred into the recipient bacterium in a 5'-terminus to 3'-terminus direction. The remaining strand is replicated, either independent of conjugative action (vegetative replication, beginning at the *oriV*) or in concert with conjugation (conjugative replication similar to the rolling circle replication of lambda phage). Conjugative replication may necessitate a second nick before successful transfer can occur. A recent report claims to have inhibited conjugation with chemicals that mimic an intermediate step of this second nicking event (Lujan et al 2007).

If the F-plasmid becomes integrated into the host genome, donor chromosomal DNA may be transferred along with plasmid DNA (Bruce et al 2002). The certain amount of chromosomal DNA that is transferred depends on how long the bacteria remain in contact; for common laboratory strains of *E. coli* the transfer of the entire bacterial chromosome takes about 100 minutes. The transferred DNA can be integrated into the recipient genome via homologous recombination.

Some strains of bacteria with an integrated F-plasmid can be isolated and grown in pure culture. Because such strains transfer chromosomal genes very efficiently, they are called *Hfr* (high frequency of recombination). The *E. coli* genome was originally mapped by interrupted mating experiments, in which various Hfr cells in the process of conjugation were sheared from recipients after less than 100 minutes (initially using a Waring blender) and investigating which genes were transferred (Hanahan et al 1991).

The nitrogen fixing *Rhizobia* are an interesting case, wherein conjugative elements naturally engage in inter-kingdom conjugation (Pan et al 1995). Such elements as the *Agrobacterium* Ti or Ri plasmids contain elements that can transfer to plant cells. Transferred genes enter the plant cell nucleus and effectively transform the plant cells into factories for the production of opines, which the bacteria use as carbon and energy sources. Infected plant cells form crown gall or root tumors. The Ti and Ri plasmids are thus endosymbionts of the bacteria, which are in turn endosymbionts (or parasites) of the infected plant.

Conjugation is a convenient means for transferring genetic material into a variety of targets. In the lab, successful transfer has been reported from bacteria into yeast (Heinemann & Sprague 1989), plants, mammalian cells (Waters 2001), and isolated mammalian mitochondria (Yoon & Koob 2005). Conjugation has advantages over some other forms of genetic transfer for engineering purposes, namely minimal disruption to the target's cellular envelope and the ability to transfer relatively large amounts of genetic material. In plant engineering, *Agrobacterium*-like conjugation is complementary to other standard vehicles, such as tobacco mosaic virus (TMV). While TMV is capable of infecting many plant families, these are primarily herbaceous dicots. *Agrobacterium*-like conjugation is also primarily used for dicots, but monocot recipients are not uncommon.

3. Bacterial Transduction. Transduction is the process by which DNA is transferred from one bacterium to another by a virus. It also refers to the process whereby foreign DNA is introduced into another cell via a viral vector. This is a common tool used by molecular biologists to stably introduce a foreign gene into a host cell's genome.

When bacteriophages (viruses that infect bacteria) infect a bacterial cell, their normal mode of reproduction is to harness the replicational, transcriptional, and

translation machinery of the host bacterial cell to make numerous virions, or complete viral particles, including the viral DNA or RNA and the protein coat (Figure 3).

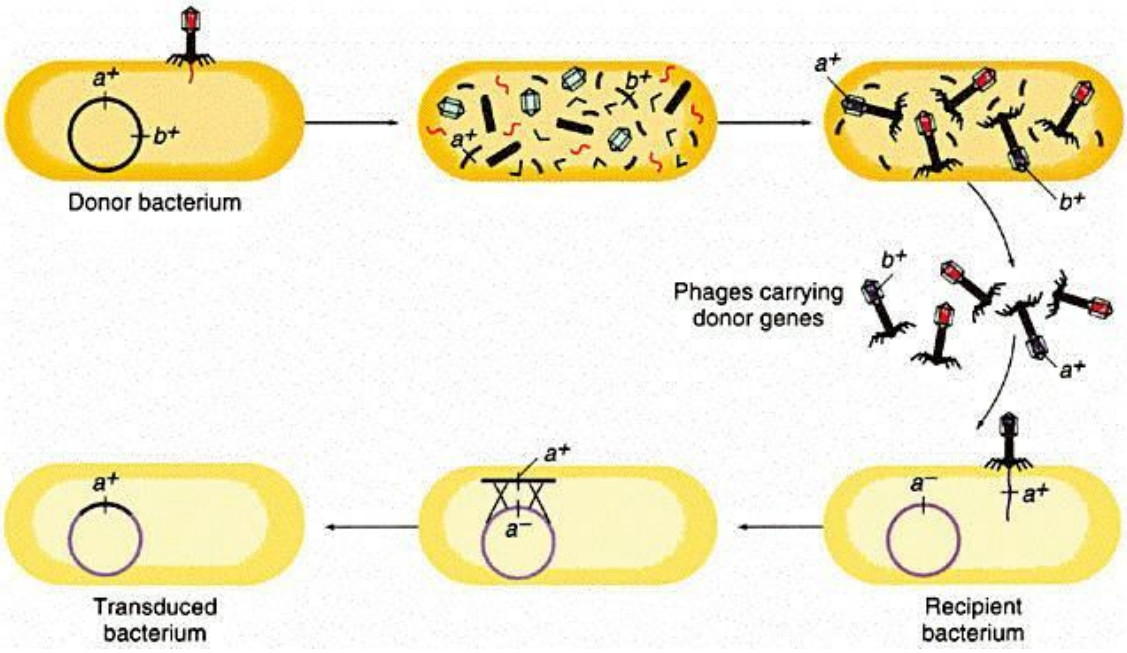

Figure 3. The mechanism of generalized transduction (***)

Transduction happens through either the lytic cycle or the lysogenic cycle. If the lysogenic cycle is adopted, the phage chromosome is integrated into the bacterial chromosome, where it can remain dormant for thousands of generations. If the lysogen is induced (by UV light for example), the phage genome is excised from the bacterial chromosome and initiates the lytic cycle, which culminates in lysis of the cell and the release of phage particles. The lytic cycle leads to the production of new phage particles which are released by lysis of the host.

The packaging of bacteriophage DNA has low fidelity and small pieces of bacterial DNA, together with the bacteriophage genome, may become packaged into the bacteriophage genome. At the same time, some phage genes are left behind in the bacterial chromosome.

There are generally three types of recombination events that can lead to this incorporation of bacterial DNA into the viral DNA, leading to two modes of recombination (Perales-Graván et al 2009).

Generalized transduction may occur in two main ways, recombination and headful packaging. If bacteriophages undertake the lytic cycle of infection upon entering a bacterium, the virus will take control of the cell's machinery for use in replicating its own viral DNA. If by chance bacterial chromosomal DNA is inserted into the viral capsid used to encapsulate the viral DNA, the mistake will lead to generalized transduction.

If the virus replicates using "headful packaging", it attempts to fill the nucleocapsid with genetic material. If the viral genome results in spare capacity, viral packaging mechanisms may incorporate bacterial genetic material into the new virion. The new virus capsule now loaded with part bacterial DNA continues to infect another bacterial cell. This bacterial material may become recombined into another bacterium upon infection (Clarck 2005).

When the new DNA is inserted into this recipient cell it can fall to one of three fates:

- The DNA will be absorbed by the cell and be recycled for spare parts.
- If the DNA was originally a plasmid, it will re-circularize inside the new cell and become a plasmid again.

- If the new DNA matches with a homologous region of the recipient cell's chromosome, it will exchange DNA material similar to the actions in conjugation.

This type of recombination is random and the amount recombined depends on the size of the virus being used.

The second type of recombination event is called *specialized transduction* and occurs as a result of mistakes in the transition from a virus lysogenic to lytic cycle. If a virus incorrectly removes itself from the bacterial chromosome, bacterial DNA from either end of the phage DNA may be packaged into the viral capsid (Hartwell et al 2000). Specialized transduction leads to three possible outcomes:

- DNA can be absorbed and recycled for spare parts.
- The bacterial DNA can match up with a homologous DNA in the recipient cell and exchange it. The recipient cell now has DNA from both itself and the other bacterial cell.
- DNA can insert itself into the genome of the recipient cell as if still acting like a virus resulting in a double copy of the bacterial genes.

Example of specialized transduction is λ phages in *Escherichia coli*.

Final Remarks. Bacteria can transfer genes from one strain to another by three different mechanisms: transformation, conjugation and transduction, these events showing the universality of sexuality in the living world. Besides genetic recombination in bacteria, recent evidences of genetic recombination in some superior animals, such as: fish (Petrescu-Mag & Bourne 2008; Petrescu-Mag 2009), birds (Pricop 2009), mammals (Henke et al 1993) and even humans (Rappold 1993), at the sex-chromosomes level support the 'gene concept of sex determination' as a general view of sex-determination.

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